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Mycorrhizae, Water Relations, Growth, and Nutrient Uptake of Geranium Grown under Moderately High Phosphorus Regimes

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Abstract. **To determine the role of endomycorrhizae on water relations and plant growth of geraniums under nonrate-limiting soil P conditions, hybrid seedlings (*Pelargonium* X*hortorum* Bailey cv. Cherry Glow) were planted in moderately high P (40 ppm) media, grown under high (−0.4 MPa) or low (−1 MPa) soil water potential (Ψ_s), and either inoculated with VA mycorrhizal fungi [*Glomus mosseae* (Nicol. & Gerd.) and *G. fasciculatus* (Thaxt. sensu Gerd.) Gerd. & Trappe] (VAM) or left as noninoculated controls. Geraniums grown under high moisture had greater shoot growth, more advanced floral development, and higher P uptake than the low moisture plants. Mycorrhizal plants under high moisture conditions had higher P levels than noninoculated plants, while mycorrhizal plants under low moisture regimes had greater shoot growth, more advanced floral formation, and greater N uptake than noninoculated plants. Xylem water potential in leaves (Ψ_L) was lower under −1 MPa than −0.4 MPa Ψ_s moisture regimes and lowest in mycorrhizal geraniums grown under −1 MPa Ψ_s. One hundred minutes after plants had recovered from water stress, the greatest change in Ψ_L was recorded for mycorrhizal geraniums acclimatized to low moisture regimes. Geraniums under low moisture regimes are more mycorrhizal-dependent. Total estimated root length and root water conductivity were lower with mycorrhizal geraniums under high water and P regime. Under water stress, the larger mycorrhizal geraniums have greater total water demands than controls. Consequently, mycorrhizal geraniums stressed more rapidly yet more efficiently recovered from water deficits. Data suggests that VAM plants acclimate more efficiently to water stress because of more frequent or extreme drought.**

There is considerable pressure on the ornamental horticulture industry to produce crops more efficiently under reduced water and fertility regimes to compensate for future government regulations on water usage and nursery runoff contamination. Manipulating the symbiotic association of mycorrhizal fungi on plant growth could lead to more efficient management systems for producing ornamental crops.

Mycorrhizal fungi enhance the growth and development of many plant species (10, 12) and their role in plant nutrition has been well-documented (6, 11, 18). Mycorrhizal fungi also have been shown to improve plant water relations (3, 7, 9, 12, 16), but the mechanism(s) by which mycorrhizal fungi influence plant water status is not well understood. Mycorrhizae may increase plant water uptake by exploiting larger soil volumes, may avoid drought by maintaining a soil-root continuum (15), or may lower stomatal resistance through regulation of ABA/cytokinin levels (3, 4, 8).

Phosphorus levels influence mycorrhizal effects on plant water status (7, 12, 15, 17). Mycorrhizal soybeans and clover under low P had higher root conductivity due to increased nutrient levels (7, 17), but citrus has shown no difference (8). Subsequent studies on drought stress recovery showed that mycorrhizal citrus seedlings had lower root conductivity, which may have been due to increased water stress brought about by higher water demands of larger mycorrhizal plants (9). Root conductivity under high P was the same in mycorrhizal and control soybean plants.

Objectives of this research were: 1) to determine the role of endomycorrhizae on water relations, growth, and nutrition of seedling geraniums under nonrate-limiting soil P conditions (40 ppm); 2) to determine the ability of mycorrhizal seedlings to recover from water stress; and 3) to determine the feasibility of producing mycorrhizal geraniums under reduced and more efficient irrigation regimes.

Materials and Methods

Inoculum of vesicular-arbuscular mycorrhizal fungi (VAM) *Glomus fasciculatus* and *G. mosseae* were cultured in containers as described previously by Strong and Davies (18). Geranium (*Pelargonium* X*hortorum* cv. Cherry Glow) seeds were germinated in sterilized vermiculite and 150 uniform seedlings were selected.

Expt. 1. Plant growth and nutrition. A randomized, complete block design was established to determine the influence of high (−0.4 MPa) and low (−1 MPa) Ψ_s on the growth and nutrient uptake of mycorrhizal and noninoculated geranium seedlings. The 4 treatments included high- and low-moisture regimes with or without mycorrhizal fungi. There were 5 replications with a total of 25 plants/treatment. One hundred geranium plants were grown in 12-cm, standard clay containers filled with steam-sterilized medium composed of 4 builders sand : 3 sandy loam : 1 composted cow manure, (by volume), with a P level of 40 ppm (Bryl extraction). Fifty grams of each VAM inoculum or 50 g of the mycorrhizal-free control was added to each container. Gypsum resistance blocks were treated according to manufacturer's instructions (Beckman) and positioned in the center of one container from each replication. Calibrated readings were

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taken with a Bouyoucos moisture meter (Model BN-2A) to monitor soil moisture levels.

Plants were established in a glasshouse with a maximum irradiance of 800 μmol $s^{-1}m^{-2}$ at 400 to 700 nm and grown for 91 days at night temperatures of 18° ± 2°C, and ambient day temperatures of 20° or higher. Plants were watered to saturation with distilled water when low (−1.0 MPa) or high (−4 MPa) Ψ_s regimes were reached. The experiment was terminated after 91 days and shoot height, width, and fresh and dry weight recorded. Total shoot N and P were analyzed with a Technicon Autoanalyzer (19). Floral development was determined on a scale from 1 to 5 with 1 = prebloom, 2 = first observable bloom, 3 = partial or half bloom, 4 = full bloom, and 5 = postfull bloom. Twenty-five, 1-cm root segments were selected randomly from each plant and VAM infection was determined using the techniques of Phillips and Hayman (14) and Bevege (5). All inoculated plants had 79% to 95% colonization.

Expt. 2. Drought stress and recovery. Leaf water potential (Ψ_L) was measured in 10 plants from each of the above treatments to determine water stress recovery rates. Water was withheld from the 40 geranium plants until −1 MPa_{Ψ_s} was recorded. Initial Ψ_L was measured between 1000–1400 HR with a plant water status console (Soil Moisture Equipment Corp). Soil then was watered to saturation and Ψ_L was recorded after 100 minutes, at which time geraniums had recovered from stress. The difference between Ψ_L measurements was used as an estimate of potential water stress recovery.

Expt. 3. Root hydraulic conductivity. Fifty additional geranium seedlings were grown in 20-cm-high × 7-cm-diameter PVC pipe in high P media described in expt. 1 to determine the effect of VAM on root water conductivity. Half of the seedlings were inoculated with combined VAM inoculum and half with roots and medium from the mycorrhizal-free control. All plants were grown under high-moisture regimes (−0.4 MPa Ψ_s) for 60 days. Plants were watered to saturation prior to measurements to eliminate possible soil conductivity differences among container medium. Geranium stems were decapitated 4 cm above the container; containers were placed in the pressure bomb with the cut end of the stem protruding through the sealing knob so that a tight seal was ensured. Pressure was applied at 2 MPa s^{-1} and initial exudation was determined. Pressure was increased gradually to 50 MPa and held for 5 min. Total exudate was determined by attaching a graduated, 5-ml pipette to the cut stem with a small piece of clear plastic tubing. Intact root systems then were washed, dried, and cut into 1-cm segments. Total root length was estimated by constructing a grid system with 1-cm squares and randomly dispersing the 1-cm root segments on the grid using techniques of Ambler (4) and Newman (13). Six counts were made on the grid for each plant and then were averaged and multiplied by the number of rows to estimate total root length. Percentage of mycorrhizal colonization was assessed (14, 16).

Results and Discussion

Plant growth and nutrition. Most reports on plant–mycorrhizal water relations have been done with soil media deficient in P (17). Consequently, the higher P levels found in mycorrhizal plants may have increased root osmotic potential due to a direct nutrient flow mechanism (7). However, this research was designed to determine the role of VAM on water relations and plant growth under more typical commercial soil P conditions. Geraniums grown under high moisture were found to have greater shoot growth, more advanced floral development, and higher P uptake than low-moisture-grown plants (Table 1). Mycorrhizal plants under high-moisture regimes had greater P uptake than noninoculated plants. Mycorrhizal plants under low-moisture regimes had greater shoot growth, floral development, and N uptake than noninoculated geraniums.

Drought avoidance may have occurred through external mycorrhizal hyphae which increased the total root system surface area (15). Consequently, the increased root-mycelial absorption area of geraniums may have enabled the extraction of soil moisture at lower water potentials. Mycorrhizal plants under water stress had increased N uptake in this study, which raises the possibility of an enhanced, direct-flow nutrient mechanism since the mycelia of the colonized roots may enhance the supply of H_2O and ions (NO_3) transferred by mass flow, as with *Bouteloua* (3) and *Trifolium* (7).

There was no difference in P uptake between low-moisture regime mycorrhizal and noninoculated geraniums, despite growth differences (Table 1). Highest P was recorded in nonstressed mycorrhizal seedlings (Table 1). Even though no growth differences occurred among high-moisture regime plants, mycorrhizal geraniums have the capacity to absorb greater P. Geranium is more mycorrhizal dependent under low-moisture regimes.

Table 1. Effects of mycorrhizae and high (−0.4 MPa_{Ψ_s}) and low (−1 MPa_{Ψ_s}) soil moisture regimes on growth, floral development, and nutrient status of geranium, evaluated 91 days after planting.

Treatment	Height (cm)	Width (cm)	Shoot fresh wt (g)	Shoot dry wt (g)	N (%)	P (%)	Floral developmental status
High moisture, no mycorrhizae	24.04 a[z]	25.64 a	71.85 a	8.09 a	1.48 ab	0.37 b	3.68 a[y]
High moisture, plus mycorrhizae	24.12 a	25.80 a	64.22 b	7.79 a	1.57 a	0.43 a	3.60 a
Low moisture, no mycorrhizae	15.56 c	19.00 c	33.71 d	3.45 c	1.40 b	0.27 c	1.40 c
Low moisture, plus mycorrhizae	18.96 b	24.16 b	45.20 c	4.73 b	1.60 a	0.29 c	2.64 b

[z]Mean separation within columns by Duncan's multiple range test, 5% level.
[y]Floral development scale ranged from 1 to 5 with 1 = prebloom, 2 = first observable bloom, 3 = partial bloom, 4 = full bloom, and 5 = postfull bloom; n = 25.

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Drought stress and recovery. Low soil moisture regime mycorrhizal geraniums were under greater water stress and recovered more efficiently than noninoculated plants. Ψ_L was lower under low compared to high soil moisture, and was lowest in mycorrhizal geraniums under low moisture regimes (Table 2). Greater recovery from water stress occurred with geraniums acclimatized to low-moisture regimes, and in mycorrhizal than in noninoculated plants. Highest change in Ψ_L was recorded in mycorrhizal plants acclimatized to low-moisture regimes (Table 2).

Larger mycorrhizal geraniums have greater total water demands under water stress than nonmycorrhizal plants and thus mycorrhizal plants underwent and more efficiently recovered from water stress. This also was documented with *Citrus* (9) and *Trifolium* (7) grown in low soil P. The Ψ_s and hence Ψ_L at which leaves closed stomates was much lower in *Trifolium* (7). Regulation by stomatal control may be a mechanism for greater drought tolerance of mycorrhizal plants (3, 7, 8). Stomatal regulation may be attributable to a secondary response to altered nutrition or cytokinin-ABA phytohormone balance (1, 2, 3, 8). Direct drought avoidance in this experiment also may have occurred because of fungal hyphal and mycelial strands increasing the plant root density and thus helping to maintain the soil-root continuum.

Root hydraulic conductivity. Mycorrhizal geraniums grown in a high-P-containing media and modified PVC containers under high moisture regimes, had greater pressure of initial exudation and lower total estimated root length and root conductivity than noninoculated geraniums (Table 3). Root length of mycorrhizal plants has been reported to be greater (7), equal (9), or less than (3) controls. Decreased root length of mycorrhizal geranium plants may indicate increased root efficiency since a relatively smaller root system is supporting a larger shoot system. No root sampling system has been devised yet to determine satisfactorily the hyphal extension of the entire root system; however, all reports are in agreement that an increased shoot/root ratio occurs with mycorrhizal plants (7, 9, 10, 12).

We found mycorrhizal geraniums to be under greater water stress which was supported by lower Ψ_L and decreased hydraulic water conductivity (increased root resistance) (Tables 2, 3). Root resistance in mycorrhizal plants under low P soil conditions has been reported lower (17, 18), equal to under high soil P (7), or greater (9, 16) than noninoculated controls.

In conclusion, root systems of the larger, container-grown mycorrhizal geraniums more effectively deplete soil water even to the point of causing greater water stress. VAM geraniums under low irrigation regimes acclimate more efficiently to water stress because they are exposed to more frequent and extreme drought. This is of importance for reducing the postharvest stress that is typical of bedding plant marketing conditions. Even though VAM increased P under high-moisture regimes, it is much too simplistic to suggest that increased P levels in VAM plants are the primary factor in VAM plants' improved water relations (17). Hormonal regulation and/or direct drought avoidance by greater maintenance of the soil-root continuum are more probable mechanisms for improved water relations (15). This research further shows the feasibility of growing geraniums more efficiently under reduced water regimes through the use of mycorrhizal symbiosis.

Table 2. Leaf water potential (Ψ_L) of mycorrhizal and noninoculated geranium plants under high (-0.4 MPa_{Ψ_s}) and low (1.0 MPa_{Ψ_s}) soil moisture regimes, measured before and 100 min after watering.

Treatment	Leaf water potential: MPa before watering, 0 min	MPa, 100 min after watering	Total change (MPa)
High moisture, no mycorrhizae	−0.63 a[z]	−0.59 b	0.05 dNS[y]
High moisture, plus mycorrhizae	−0.65 a	−0.50 a	0.15 cS
Low moisture, no mycorrhizae	−1.00 b	−0.64 b	0.36 bS
Low moisture, plus mycorrhizae	−1.29 c	−0.63 b	0.66 aS

[z]Mean separation within columns by Duncan's multiple range test, 5% level.

[y]Mean separation within rows by Duncan's multiple range test. NS = change in Ψ_L not significant, S = change in Ψ_L significant, 5% level; n = 10.

Table 3. Root hydraulic conductivity of mycorrhizal and nonmycorrhizal geranium plants under high (-0.4 MPa_{Ψ_s}) moisture regimes and moderately high P regimes (40 ppm).

Treatment	Pressure of initial exudation (MPa)	Total exudate (ml)	Total estimated root length (cm)	Ml exudate 100/cm of root length
High-moisture inoculated control	0.27	1.1[z]	1020	0.11
G. fasciculatus *G. mosseae* colonized	0.31	0.7	876	0.09
Probability F[y]	0.1%	0.1%	0.1%	0.2%

[z]Collected in 5 min at 0.5 MPa.

[y]Means determined by a general linear model procedure: n = 25.

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Biochemical Markers for *Carica papaya*, *C. cauliflora*, and Plants from Somatic Embryos of Their Hybrid

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Abstract. **Isozyme markers for glutamate oxaloacetate transaminase (GOT), superoxide dismutase (SOD), peroxidase (PER), and malate dehydrogenase (MDH) were identified for *Carica papaya* L. and the related but sexually incompatible *C. cauliflora* Jacq. These markers were used to determine the nature of somatic embryos derived from papaya ovules cultured on modified Murashige and Skoog (MS) medium 65 days after controlled pollination with *C. cauliflora*. Zymograms of plantlets from somatic embryos contained bands specific to either *C. papaya* or *C. cauliflora* (PER, GOT) and a unique band not present in the zymogram of either species (PER). Zymograms of somatic embryo-derived plantlets were distinctively different from those of either of the *Carica* species for all the enzyme systems examined. Evidence from isozyme markers indicates that somatic embryos produced from cultured papaya ovules following pollination with *C. cauliflora* may be hybrids. The isozyme banding patterns of 60 plantlets derived from somatic embryos from the same ovule were very uniform and suggest genetic uniformity among the regenerated plantlets.**

The papaya, *Carica papaya*, is one of the most widely grown fruits in the tropics. In recent years, a serious disease caused by papaya ringspot virus (PRV) has affected adversely papaya production in many parts of the world. Efforts to identify sources of resistance to PRV and to transfer this resistance to papaya have been reported (2, 8). Conover and Litz (3) found that PRV resistance in some papaya accessions from South America was conferred by a complex of genes. Monogenic resistance to PRV, which is conferred by a dominant gene, has been identified in 3 other *Carica* species, *C. stipulata* Badillo, *C. cauliflora*, and *C. pubescens* Lenne & Loch (8, 9). All of the PRV-resistant species are sexually incompatible with papaya.

Khuspe et al. (11) reported the successful *in vitro* culture of immature zygotic embryos from crosses between *C. papaya* and *C. cauliflora*. Litz and Conover (13) observed that polyembryony occurred in cultured papaya ovules derived from the same interspecific cross. The embryogenic response was highly dependent upon the maternal genotype and could not be induced in some papaya types (15). The somatic embryos could be induced to germinate and regenerated plants have been established in the greenhouse (14). These plants closely resembled the maternal papaya parent. However, because *in vitro* polyembryony occurred only rarely, histological studies were ineffective in determining the anatomical origin of the somatic embryos. If the regenerated plants are interspecific hybrids, they could be used to transfer PRV resistance from *C. cauliflora* to papaya.

Biochemical markers such as isozymes have been used widely to identify both sexual and somatic hybrids (1, 5, 6, 23, 24). Isozyme bands have been used also to characterize the amount or kind of variability present in plant populations produced through

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